Assessing the Potential of Diverse Forage Mixtures to Reduce Enteric CH_4 Emissions

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Abstract

Enteric methane (CH_4) is a main source of agriculture-related greenhouse gasses. Conversely, pasture is increasingly demanded by customers due to both perceived and real benefits regarding animal welfare, environmental aspects and product quality. However, if implemented poorly, CH_4 emissions can increase, thus contributing to climate change. One promising option to reduce enteric CH_4 emissions are plant specialized metabolites (PSM), and particularly tannins. Consequently, we conducted two complementary experiments to determine to what extent enteric CH_4 emissions can be reduced, and how this affects milk yields: a) an *in vivo* experiment with grazing Jersey cows, where CH_4 emissions were quantified using the SF_6 tracer technique, and b) an *in vitro* experiment using the Hohenheim gas test. In the *in vivo* experiment, a binary mixture consisting of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) was compared against a diverse mixture consisting of eight species, including birdsfoot trefoil (*Lotus corniculatus*), and salad burnet (*Sanguisorba minor*). In the *in vitro* experiment, the eight species from the *in vivo* experiment were combined in binary mixtures with perennial ryegrass in increasing proportions, to determine the mitigation potential of each species. Results show an increase in milk yield for the diverse mixture, although this is also accompanied by higher CH_4 emissions. Nevertheless, these emissions are lower across both mixtures, when compared with similar trials. This is probably due to a very high digestibility of the ingested forage. With the *in vitro* experiment, we were able to confirm a substantial potential for CH_4 reduction when including species rich in PSM. However, those forbs with the higher anti-methanogenic potential were only present in minor proportions in the pasture. Hence, further research will be required on how to increase the share of the bioactive species with lower competitiveness and confirm their potential *in vivo*.

Introduction

Enteric methane is a by-product of ruminal fermentation of fibrous feed, that allows the ruminal ecosystem to dispose of the metabolic hydrogen produced during microbial metabolism (McAllister and Newbold, 2008). This is problematic for two reasons: a) methane emissions represent an energy loss, contributing to an inefficient digestion, and b) enteric methane represents 44% of anthropogenic methane emissions, thus contributing to climate change (Gerber et al., 2013). However, despite this environmental impact, grazing ruminants have the evolutionary advantage to produce food (meat and milk) from grass, and otherwise marginal lands may be used to produce edible energy and protein for humans. Additionally, grazing systems are not only the most cost-effective ruminant production system, but also contribute to soil carbon sequestration (hence contributing to climate change mitigation), biodiversity, and animal welfare (Dillon et al., 2005). Therefore, grazing dairy systems appear to be beneficial for an environmentally friendly and economically viable production (Peyraud et al. 2010; Thomet, et al. 2011). Additionally, the use of pastures has been shown to be positively linked to consumer preference (Kühl et al., 2017; Weinrich et al., 2014). With methane currently being one of the biggest environmental burdens from dairy and beef production, mitigation strategies for the reduction of the GHG emissions are needed to maintain the benefits of grazing-based ruminant production systems, while alleviating the negative impact on the climate.

One promising option to reduce enteric methane emissions are condensed tannins (CT). In the rumen, CT bind with dietary protein, thus protecting it from enzymatic hydrolysis and reducing enteric methane production (Jayanegara et al., 2012). These complexes dissociate due to changes in pH in the abomasum or the small intestine (Wang et al., 1996), thereby releasing the protein for post-ruminal digestion. As a result, including species into the animals diet that contain plant secondary metabolites (PSM), and particularly CT, is hypothesized to reduce methane emissions and increase nutrient use efficiency. This solution is particularly promising as improving species-richness can improve the swards yields, drought tolerance and reduce nutrient input requirements (Jing et al., 2017). However, increasing plant diversity in swards strongly influences the nutritive value of the herbage. Consequently, the aim of this study was a) to determine to what extent enteric CH_4 emissions can be reduced by increasing pasture diversity with CT-containing forage species, and how this affects milk yields (Experiment 1) and b) determine the required shares of each species in a mixture with ryegrass to reduce methane emissions significantly (Experiment 2).

Methods and Study Site

Experiment 1: In vivo study determining the potential of increased pasture diversity

This experiment was carried out between March and August of 2019 at Kiel University‘s research farm "Lindhof" (Kiel, Germany. 53°40´N, 10°35´E). Twenty-four multiparous lactating Jersey cows were split in two groups based on the following factors (mean ± SD): pre-experimental milk yield (17.4 kg/day ± 2.7), days in milk (DIM) (45 d ± 28), live weight (LW) (448 kg ± 51) and parity (3 ± 1), and were grazing either a binary mixture (*Lolium perenne* (Lp) and *Trifolium repens* (Tr)), or a diverse mixture (*Lp, Tr, Trifolium pratense* (Tp), *Lotus corniculatus* (Lc)*, Sanguisorba minor* (Sm)*, Cichorium intybus* (Ci)*, Plantago lanceolata* (Pl) and *Carum carvi* (Cc)). The experiment was conducted using a cross-over design and two main periods (P1 and P2) with two-subperiods each. Five weeks prior to the first measurement period, all animals started grazing on the same paddocks, which were a selection of both the binary and diverse mixture to pre-adapt the rumen fauna to both pastures. Each sub-period then consisted of 4 days of a pasture-specific adaptation period, followed by 4 days of measurements. Swards were strip-grazed at a daily herbage allowance of 18 and 14 kg/cow/day for P1 and P2, respectively. The target residue was 4 kg DM/cow/day. Throughout the study, cows were milked twice a day (0600 and 1600 h) and individual milk yield was recorded. Milk composition (protein, fat and lactose) was determined daily from morning and afternoon milk samples. Energy corrected milk (ECM) was estimated according to Sjaunja (1990) and enteric methane emissions were measured using the SF_6 tracer technique reported by Johnson & Johnson (1995) as adapted by Gere & Gratton (2010) for a 4-day collection period. Herbage mass and botanic composition was estimated by cutting ten randomly chosen quadrats of 0.25 m^2 per plot to a height of 4 cm.

Experiment 2: In vitro study analyzing defined mixtures of grass and legumes/herbs

In this experiment, ten species (the aforementioned eight from the diverse mixture, as well as *Lotus pedunculatus* (LoP) and *Sanguisorba minor* (Sm)) were combined in binary mixtures. *Lolium perenne* was used as fixed partner, while the 9 other species were added in six increments from 0 to 100%. Samples of each species for the *in vitro* analysis were collected separately from the diverse sward used in Experiment 1, before grazing, in early spring 2018, when the sward was in late vegetative stage. Approximately 300 g of fresh plant material was cut to a residual height of 4 cm. Samples were immediately frozen and stored at -27 °C. Before analysis, samples were freeze-dried to a constant weight and milled to a particle size of 1 mm.Total gas production and methane production of these forage mixtures were determined using the Hohehnheim gas test (HGT) following the procedure as stated by Menke and Steingass (1988). Forage quality parameters of all samples analysed in Experiment 1 and Experiment 2 were determined by near-infrared reflectance spectroscopy (NIRS) with a NIRSystems 5000 monochromator (FOSS, Silver Spring, USA). Mathematical evaluation of the spectra was performed using the Modified Partial Least Squares method (WinISI software version 3, Infrasoft International, USA). The percentage of digestible organic matter (DOM) was calculated using the enzymatic soluble organic matter (ELOS) and the enzymatic insoluble organic matter (EULOS). Calibration and validation were based on sample subsets of perennial ryegrass, legumes and forage herb species, which represented the whole spectral and chemical variability.

Results

Experiment 1

The chemical composition of both herbage mixtures on offer showed generally a high quality, despite decrements over time. Mean values for OM digestibility of both mixtures were between 0.78 and 0.88, and the mean energy concentration -as NEL- was in the range of 6.7 to 7.7 MJ/kg DM, throughout P1 and P2. On average across periods, OM digestibility and energy content of the binary mixture were 3.5% greater (P<0.001) compared to the diverse mixtures. In contrast, across both periods, crude protein (15.0 g/kg DM) and ADF contents (19.7 g/kg DM) of the binary mixtures were lower (P<0.001) when compared to the diverse mixtures (17.8 and 23.1 g/kg DM, for crude protein and ADF) across both periods. *Lolium perenne* was the predominant species in both mixtures. When comparing the botanical composition of the residual swards after grazing with that of the offered forage, a positive selection by grazing animals for the herb species in diverse mixtures could be identified, with herb shares having decreased by 60%, on average for P1 and P2, when compared to the offered herbage. The forage use efficiency (FUE), was similar between treatments over both periods, yet with a non-significant tendency of a greater FUE in the diverse pasture (63 % vs 71% FUE on average, for binary and diverse mixtures, respectively, across P1 and P2). Averaged across both periods, milk yields (22.3 kg and 21.0 kg milk/cow/day for diverse and binary mixture, respectively) and ECM yields (26.9 and 25.7 kg ECM/cow/day for diverse and binary mixture, respectively) increased ($P<0.001$) when cows grazed diverse mixtures. However, daily methane emissions increased in diverse pastures as well, and mean values were 18% greater ($P<0.01$) in diverse pastures, with mean values of 221 and 260 g CH_4/day for binary and diverse mixtures, respectively . Mean values for methane emissions per kg ECM were 11% greater ($P<0.01$) in diverse, compared to binary mixtures, with 9.8 and 8.8 g CH_4/kg ECM on average across P1 and P2, respectively. Over time, the methane intensity generally increased and was 11% lower ($P<0.05$) in P1 compared to P2, with mean values of 8.8 and 9.9 g CH_4/kg ECM, respectively.

Experiment 2

In vitro, most species showed a linear tendency to reduce methane emissions with increasing legume/herb share, although the extent of this reduction varied significantly with the partner species. The greatest methane reduction was observed for Sm and LoP (-24 %, on average). The most common forage legumes, *Tr* and *Tp*, did not decrease methane emissions at all compared to *Lolium perenne*. Simultaneously, there was a linear reduction in total gas production (i.e. OM digestibility) in all species, and, similarly to methane production, the species with the greatest reduction were Sm and LoP (-28%, on average), followed by Tr and Tp (-21%, on average), Lc (-20%, on average), Ci and Cc showed the lesser reduction in toal gas (-13%, on average). These differences in methane suppression potential were in agreement with the tannin composition, as the highest tannin concentrations were observed in Sm (47.6 mg/g DM) and LoP (20.2 mg /g DM), whereas all other species had only minor tannin concentrations of less than 4 mg / g DM. However, while Sm contained entirely hydrolysable tannins (HT), LoP contained condensed tannins (CT). Sm also containted the greatest energy contents with 7 MJ NEL / kg DM and an OM digestibility of 89%. These were lower in LoP at 6.1 MJ NEL/ kg DM and an OM digestibility of 80%. *Lolium perenne,* also exhibited a high forage quality, with 6.5 MJ NEL / kg DM and an OM digestibility of 85%.

Discussion

The *in vitro* experiment results showed substantial potential to reduce methane production, when including species rich in PSM. *Lotus pedunculatus* and *Sanguisorba minor* are of particular interest, since they showed the greatest reduction potential. However, the simultaneous reduction in OM digestibility (i.e. reduction in total gas production) when increasing the shares of these species in the diet might be an issue. Nevertheless, the cows grazing the diverse mixture in the *in vivo* experiment had excellent milk yields, comparable to the milk yields of Jerseys fed TMR diets with 32% concentrate *ad libitum* (Olijhoek et al., 2018). The fact that the cows in the present study had milk yields similar to those of Jersey cows fed TMR with a high proportion of concentrates, despite slightly lower body weights (with 442 and 469 kg, respectively) indicates that forage of excellent quality was available on the pastures in general, as well as the cows having a high genetic potential for milk production. The high forage quality and genetic potential of the Jersey cows as illustrated in the milk yield, can also explain, at least partially, the very low CH_4 emissions and particularly the methane intensities. Generally, the CH_4 emissions in the present study of 221-260 g CH_4/cow/day, were similar to values reported previously for Jersey cows, ranging from 258 to 321 g CH_4/day (Münger and Kreuzer, 2006; van Wyngaard et el., 2018). However, when compared to cattle with similar body weight, these emissions are substantially lower than the ~ 400 g CH_4/day reported by Jonker et al., 2019. Additionally, due to the very high milk yields, the emissions per unit of milk were low. The reasons for increased CH_4 emissions from cows that grazed the diverse swards are likely to be a combination of two factors. Firstly, the greater DMI (as indicated by the greater FUE) of herbage from the diverse mixture sward, and secondly, the lower OM digestibility of the diverse sward, which is to be expected, due to the share of herbs rich in structural carbohydrates, such as ribwort plantain, and the temporal asynchrony between species and the corresponding difficulty in utilizing all species under optimal conditions, thus resulting in increased proportions of stem fractions in many species (Bruinienberg, 2002). However, these forbs with the higher anti-methanogenic potential were only present in minor proportions in the pasture, probably because of a lack of competitiveness in a system with high grazing pressure. This might explain that their shares did not suffice to reduce methane emissions compared to the binary mixture in the *in vivo* study, contrary to the *in vitro* findings. Hence, while the overall very low emissions and high milk yields have identified the suitability of the grazing system for milk production with low methane emissions, with regards to the PSM containing forage plants further research is needed to identify whether different mixture strategies or lower grazing intensities can improve their potential for grazing systems with dairy cows.

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